Low Temperature Expression of an Ice Binding Protein in an Antarctic Bacterium

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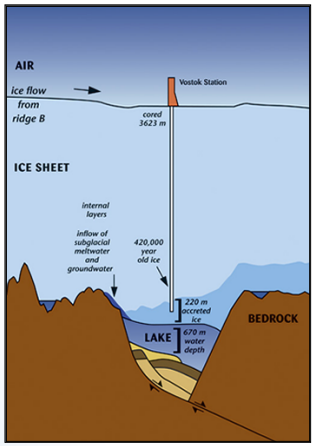
Abstract

A bacterium recovered from the Vostok ice core was found to possess an Ice Binding Protein (IBP) homologous to those described in some cold-adapted marine bacteria, molds, and diatoms (1). During the phase transition of water to ice, the presence of the secreted IBP alters the structure of the ice crystals and prevents recrystallization which is often times extremely damaging to the cell membrane. The IBP is suspected to provide a phenotypic advantage under frozen conditions by preventing the damage associated with ice formation and recrystallization as well as conserving the liquid veins within the ice. To explore the temperature-dependent regulation of the IBP, Reverse Transcription-PCR was used to examine gene expression at various temperatures. Expression of the IBP gene was detected at 4°C, but not at 10°C or warmer temperatures. These results provide evidence for a low temperature stress response that is distinct from the classical bacterial cold-shock response.

Introduction

Lake Vostok is the largest subglacial lake yet identified in the world with a surface volume of approximately 14,000 km2. Located about 4 km under the ice sheet, it has been isolated from the atmosphere for an estimated 15 million years. Lake Vostok has been the site of many drilling projects, some of which have extended down to the accreted ice above the lake itself. This ice is built not from precipitation but the freezing of lake water, preserving in it clues to the lake's geochemical and biological composition. It is expected that the lake itself will be sampled in the next couple of years (2).



Schematic depicting the Lake Vostok ice core and the structure of the lake itself.
Image from: http://www.astro.yale.edu/chunter/presentations/cty/earth-habitat.html

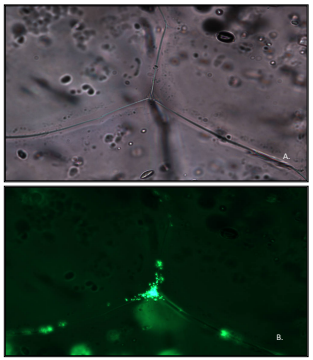
References

1.Raymond, James et al. 2008 .A bacterial ice-binding protein from the Vostok ice core. Extremophiles. 12:713–717.
2.Christner, Brent et al. 2006. Limnological conditions in Subglacial Lake Vostok, Antarctica. Limnology and Oceanography. 51(6):2485-2501.

Microorganisms in ice

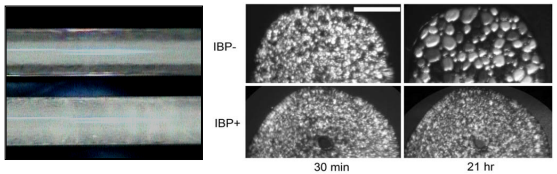
During ice formation, solutes are excluded from the ice lattice creating a complex network of high salinity water veins around the individual crystals. These veins have been found to be a possible habitat for microbial life because they too are excluded into the liquid veins along with the solutes. This type of environment would allow microorganisms to survive in "solid" ice.

Cross Section of an Ice Vein at a Triple Junction



A. Micrograph of liquid water vein at a triple junction.
B. Same area under epifluorescence, with Sybr Gold stained cells in the triple junction

Recrystallization Inhibition caused by the Ice Binding Protein



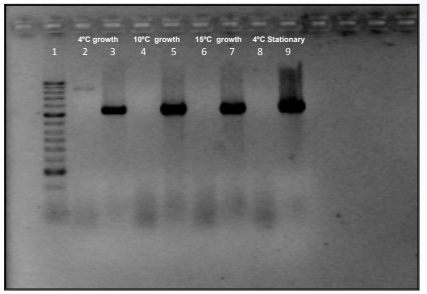
Ice crystals formation in the absence and presence of the IBP demonstrates the protein has recrystallization inhibition (RI) activity.
Left: Microcapillary tubes show the different freeze patterns of ice in the absence and presence of the IBP.
Right: In samples containing IBP, crystals were visibly inhibited. Photo by James Raymond.

Material and Methods

A pure culture of the bacterial isolate (designated 3519-10) was grow in R2 media at temperature of 15˚C, 10˚C, and 4˚C until mid-logarithmic growth, at which time cells were harvested and preserved in RNAprotect Reagent (Qiagen) to prevent degradation of RNA. The cultures were stored at -80˚C until RNA was extracted using the Rneasy Mini Prep kit (Qiagen) and according to the manufacturers protocol. Genomic DNA was removed with the addition of the optional on-column DNase (Qiagen). Oligonucleotides designed to amplify a 1.6 kb fragment of the IBP gene were used with the One-Step RT-PCR kit (Qiagen). The products were separated by electrophoresis and visualized on a 2% agarose gel stained with ethidium bromide.

Results

RNA was successfully extracted using the method described. An average of 50 µg mL^{-1} of total RNA was obtained from each extraction with consistently lower yields frm the 4˚C cells cultures.
The extraction of DNA-free RNA was difficult and several different DNase options were explored, with the best results from the On-column DNase kit.



Reverse Transcriptase PCR on RNA extracted from ell cultures grown a 4˚C, 10 ˚C, and 15 ˚C. Lane 1 contains a 2 kbp ladder. Lanes 2,4,6, and 8 contain the ice binding protein specific primers. Lanes 3, 5, 7, and 9 contain rRNA primers 348F and 1391R which act as a control for the presence of RNA.

By this assay, the ice binding protein appears to only be expressed at 4˚C, as seen with the approximately 1.6 kbp band in lane 2. All other bands indicate the presence of rRNA. In addition, the cells harvested from a 4˚C culture well into stationary phase did not appear to express significant enough amounts of the Ice Binding Protein to be detectable.

Conclusions and Future Implications

At temperatures below the freezing point of water, the phase transition of water to ice is directly associated with a variety of physical, osmotic, and metabolic stresses to the cell. Due to its ability to manipulate ice structure, the Ice Binding Protein of this bacterium could provide a distinct advantage to a species that has not experienced temperatures above the melting point of ice for ~1 million years. Previous work had shown that the IBP protein is expressed at 4ºC (1), and our results imply this regulation is likely to be at the level of gene expression. More experiments are required to further characterize the gene and protein expression of the IBP as well as similar cold temperature-expressed genes in this bacterium and others. Microbial products that bind to, inhibit, and control the size, shape, and growth of ice crystals, like the IBP, could offer new possibilities for a number of agricultural, biomedical, and industrial applications.

Acknowledgements

This work was supported by National Science Foundation grant EAR-0525567. A.A. was partially supported with a grant to the Louisiana State University College of Basic Sciences from the Howard Hughes Medical Institute Biomedical Education Program.
Special thanks to Adrian Lopez, LSU SOAR Coordinator